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TRITIUM DYNAMICS IN MICE EXPOSED TO TRITIATED WATER, TRITIATED DIET OR BOTH

Report No 88-30-K

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ABSTRACT

Juvenile female mice were exposed to tritiated water, tritiated diet or both for 56 d. The specific activity of tritium in tissue water and organic tissue of the mice was followed during and following tritium exposure. During tritium exposure, the effects of water and diet on the steady state activities of tissue water tritium and organically bound tritium were largely independent and additive. The steady state activities of tissue water tritium and organically bound tritium in mice exposed to both tritiated water and diet were essentially equivalent to the sum of the steady state specific activities of mice exposed only to tritiated water and that of mice exposed only to tritiated diet. Tissue water tritium was accumulated fairly rapidly, with half-lives of 1 to 2.6 d. In contrast, organically bound tritium was accumulated more slowly. Following tritium exposure, the loss of organically bound tritium was resolved into two distinct clearance compartments. The rate constants for the fast clearing organically bound tritium compartment were not significantly different from the corresponding rate constant for tissue water tritium, and thus likely represented the clearance of loosely bound or exchangeable organically bound tritium. The second organically bound tritium compartment was cleared much more slowly, with half-lives of 20 to 30 d, and likely represented non-exchangeable tritium.

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EXECUTIVE SUMMARY

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Biologist
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Tritium, largely in the form of tritiated water, is among the principal radionuclides released in atmospheric emissions and aqueous effluents of Ontario Hydro Nuclear Generating Stations. Following release, tritiated water will be distributed among the components of the ecosystem, including biota. Through exchange and synthesis, this tritiated water may then be incorporated into organic compounds to form organically bound tritium. In environments subject to chronic tritium release, animals will thus be exposed to tritiated water in the ambient air and water and tritiated organic compounds in the diet.

Existing models of the metabolism and dosimetry of tritium (ie, IRCP 1979) have focused on the dose received from tritiated water. However, recent studies have indicated that the inclusion of the dose from organically bound tritium may significantly increase the total radiation dose resulting from chronic tritium exposure. Accordingly, this study was initiated to define the changes in activity of tissue water tritium and organically bound tritium in mice chronically exposed to tritiated water, tritiated diet or both.

The tritium specific activity of the mice resulted from a dynamic equilibrium of three principal compartments - tissue water tritium, loosely bound or exchangeable organically bound tritium, and tightly bound, non-exchangeable organically bound tritium. During tritium exposure, the effects of water and diet on the steady activities of tissue water tritium and organically bound tritium were largely independent and additive. The steady state activities of tissue water tritium and organically bound tritium in mice exposed to both tritiated water and diet were essentially equivalent to the sum of the steady state specific activities of mice exposed only to tritiated water and that of mice exposed only to tritiated diet. Tissue water tritium was accumulated fairly rapidly, with half-lives of 1 to 2.6 d. In contrast, organically bound tritium was

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accumulated more slowly. Following tritium exposure, the loss of organically bound tritium was resolved into fast and slow clearance compartments. The rate constants for the fast clearing organically bound tritium compartment were not significantly different from the corresponding rate constant for tissue water tritium, and thus likely represented the clearance of loosely bound or exchangeable organically bound tritium. The second organically bound tritium compartment was cleared much more slowly, with half-lives of 20 to 30 d, and likely represented non-exchangeable tritium.

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To Dr. D. Mills
Director of Research

TRITIUM DYNAMICS IN MICE EXPOSED TO TRITIATED WATER, TRITIATED DIET OR BOTH

INTRODUCTION

Tritium, largely in the form of tritiated water, is among the principal radionuclides released in atmospheric emissions and aqueous effluents of Ontario Hydro Nuclear Generating Stations (OHHSD SSD-AR-86-1). Following release, tritiated water will be distributed among the components of the ecosystem, including biota. Through exchange and synthesis, this tritiated water may then be incorporated into organic compounds to form organically bound tritium (OBT) (Feinendegen 1967). In environments subject to chronic tritium release, animals will thus be exposed to tritiated water in the ambient air and water and tritiated organic compounds in the diet (Vanderplong et al. 1975).

Within the organism, tritium will be partitioned between two major pools; tritiated water within the tissues of the organism (tissue water tritium - TWT) and tritium incorporated into organic molecules as organically bound tritium. Organically bound tritium may be further segregated into an exchangeable fraction, which exchanges hydrogen freely with the cellular water, and a non-exchangeable fraction in which there is little or no exchange of hydrogen (Feinendegen 1967; Commerford 1984). Under physiological conditions, hydrogen atoms linked to oxygen, nitrogen or sulphur atoms exchange hydrogen readily with the ambient water but there is little or no exchange of hydrogen atoms linked to carbon atoms (Commerford 1984).

The dynamics of tissue water tritium in mammals are well established. Tissue water tritium in mammals exposed to tritiated water rapidly equilibrates to specific activities which are 60 to 75% of the activity of the drinking water (Thompson and Ballou 1956; Richmond et al. 1962; Hatch and Mazrimas 1972). The difference between the steady state activity of tissue water tritium and drinking water is attributed to a dilution of the tissue water within the organism by metabolic and food water (Hatch and Mazrimas 1972). In general, the rate of turnover of tritiated water within the animal is inversely related to body size, with biological half-lives of approximately 1 d in mice and 9.5 d in man (Richmond et al. 1962).

The dynamics of organically bound tritium are less well defined. In rats and mice exposed to tritiated water for more than 30 days, organically bound tritium in most tissues equilibrated to specific activities which were 20 to 30% of the specific activity of the drinking water (Thompson and Ballou 1956; Hatch and Mazrimas 1972). However, fatty tissues reached higher specific activities of approximately 40% that of the drinking water. The specific activity of organically bound tritium changes much more slowly than tissue water tritium and in some tissues, the

biological half-lives of organically bound tritium may exceed 100 days (Thompson and Ballou 1956).

Although a number of studies have demonstrated that organically bound tritium may be accumulated through the diet, the relative importance of diet to the accumulation of organically bound tritium is poorly resolved. In rats or rabbits exposed to organically bound tritium in the diet, tritium activities in the organically bound tritium fraction were significantly greater than the activities of tissues of animals exposed for the same duration to tritiated water only (Rochalska and Szot 1977; Pietrzak-Flis et al. 1978). For both mice and marine snails, the ratio of hydrogen isotopes in the animal corresponded to the composition of its diet rather than the ambient water (Estep and Dabrowski 1980). In larger domestic animals exposed to tritium by water, diet or both, organically bound tritium was transferred with the milk in lactation and was also cleared more slowly than tissue water tritium (van den Hook 1986; Van Hees et al. 1986). However, the limited numbers of animals and sampling intervals in many of the above studies, precludes detailed definition of the kinetics of accumulation of organically bound tritium or the estimation of steady state activities.

A more detailed knowledge of the kinetics of accumulation of organically bound tritium is required as this form of tritium could contribute significantly to the radiation dose resulting from the release of tritium to the environment. Present models of the metabolism and dosimetry of tritium in man (ICRP 1979) have considered only the dose received from tritiated water. As greater than 99% of the tritium released to the environment is in the form of tritiated water (Butler 1980), these models may be reasonable for acute exposure. In chronic environmental exposure, however, mammals will also be exposed to organically bound tritium. Because it is cleared much more slowly than tissue water tritium, organically bound tritium may significantly increase the estimated dose resulting from exposure to tritium. In a model of the dose resulting from chronic exposure to tritiated water, the total dose increased by approximately 20% when the dose from organically bound tritium formed by exchange with tissue water tritium was included (Till et al. 1979). Inclusion of doses from ingestion of tritiated diet will further increase the total dose commitment from organically bound tritium (Crawford-Brown 1984).

This study was initiated to resolve the relative contributions of ambient water and diet to chronic accumulation of tritium in mammals. Juvenile female mice were exposed to tritiated water, tritiated diet or both for 56 d. The specific activity of tritium in tissue water and organic tissue was determined during tritium exposure and for 35 d following tritium exposure.

MATERIALS AND METHODS

Juvenile female Swiss albino mice (Mus musculus) approximately 4 wk old and 15 to 20 g wet weight, were purchased from a commercial supplier (High Oak Ranch, Goodwin, Ontario). The mice were housed, in pairs, in plastic cages (20 x 26 x 15 cm) which were covered by wire lids. The cages were placed on metal racks in two animal holding rooms with 90 cages per room. Photoperiod was regulated at 12 h light - 12 h dark, while temperature was maintained at 24.5 $\pm$ 1.5°C and the relative humidity was 35 $\pm$ 10%. The animal holding rooms were ventilated to provide for an exchange with outside air 7 times per hour.

The mice were provided with free access to water and food. Water was contained in closed plastic bottles (250 mL) fitted with 1.8 mm stainless steel sipper tubes. The diet (Rodent Laboratory Chow #5001, Ralston Purina Co, St. Louis Mo) was contained in the food hopper of the wire

cage lid. Water bottles and cages were cleaned weekly, at which time the bedding (Beta Chip, Northeastern Product Corporation, Warrensburg, NY) within the cages was also changed.

For the first week following receipt, the mice were provided with free access to unlabeled water and unlabeled diet. The mice were then exposed to tritiated water, tritiated diet or both for the next 56 d. Treatments were:

1) Tritiated Water - Mice were given free access to tritiated water of an activity of 22.9 ± 0.1 kBq/g H (2.56 ± 0.01 MBq/L, Mean ± SE n=8) and unlabeled diet.

2) Tritiated Diet - Mice were given free access to a diet containing added tritiated amino acids with an activity of 34.4 ± 0.4 kBq/g H (2.11 ± 0.02 kBq/g dry wt, n=12) and unlabeled water.

3) Tritiated Water and Diet - Mice were given free access to both tritiated water and diet at the specific activities given above.

After 56 days of exposure to tritiated water, diet or both, tritiated water and diet were removed and mice on all treatments were given free access to unlabeled water and diet for the remainder of the experiment. At 0, 1, 2, 4, 7, 14, 21, 28, 35, 42, 49 and 56 d during tritium exposure and at 1, 2, 4, 7, 14, 21, 28 and 35 d following tritium exposure, the mice were sampled. For each sample, 3 cages were selected at random from each treatment, with 5 cages sampled at the termination of tritium exposure (56 d). The mice were anesthetized with ether, killed by cervical dislocation, then frozen (-20°C) in closed plastic bags and analyzed within 1-4 weeks.

Growth of the mice was not affected by the level of tritium exposure, as the mean weights of sampled mice did not differ significantly among treatments during the experiment (Split-plot ANOVA, $p > 0.05$). The average weight of the mice increased significantly over the course of the experiment, from a mean of 23.8 ± 0.4 g (Mean ± SE, n = 9 cages) on Day 1, to 31.0 ± 0.9 g on Day 56 (n = 15) and 34.2 ± 0.6 g on Day 91 (n = 9). During the experiments, 9 mice died (1.7% mortality), with mortality apparently unrelated to treatment. In all cases, only one of the two mice in the cage died, and the other mouse survived until the cage was sampled.

The tritiated water and amino acid mixture were purchased from Amersham, Oakville, Ontario. The tritiated water was diluted from a concentrated stock and stored in 100 L reservoirs which were refilled once during the course of the experiment. The tritiated amino acid mixture contained a mixture of 15 L-amino acids, which are listed by activity in Appendix 1. Tritiated diets were prepared by mixing an aqueous solution of the tritiated amino acid mixture with the powdered commercial diet. The wet diet mixture was extruded in a food mixer, freeze dried and kept frozen in plastic bags until it was used within the next three weeks.

Tritium activities in water, diet and mice were determined by liquid scintillation counting using a Packard Tri-Carb Scintillation Counter (Model 300C). For analysis of tritiated water, duplicate samples (1 mL) were mixed with PCS II (14 mL) scintillation cocktail for determination of sample activity. For analysis of tissue water and organically bound tritium, the mice were homogenized in a tissue blender after removal of their stomach and intestines. Tissue water was then extracted from the homogenized tissues by azeotropic distillation with toluene (Moghissi et al. 1973), and the tritium activity of tissue water determined by liquid scintillation counting of duplicate samples of distillate (1 mL) mixed with PCS II (14 mL). Following the extraction of tissue water,

samples were desiccated over phosphorus pentoxide to constant weight. Duplicate samples of dried tissue (approximately 0.5 g) were then oxidized in a Packard Tri-Carb Oxidizer (Model C306). The activity of organically bound tritium in the dried tissues was determined by liquid scintillation counting of combustion water, which was collected automatically and mixed with Monophase 40 scintillation cocktail (15 mL). In earlier experiments, distillation of up to 100 g of freeze dried, tritiated diets did not yield measurable quantities of water (Ontario Hydro Research Division Report No 84-217-K), thus only organically bound tritium activity of the diets was determined.

Tritium activities are expressed as specific activities (Bq) per gram of hydrogen and are corrected for background, quench and radioactive decay. Background activities were consistently less than 1% of total activity with the exception of the unlabeled water and diet, and mice on Day 0, all of which counted at background rates. The specific activity of water samples was obtained by dividing the observed activity by the weight of hydrogen in 1 g of water at 20°C (0.1117 g).

Hydrogen content of the diets and mice was determined gravimetrically after trapping water produced in combustion of oven dried, desiccated samples (Ontario Hydro Research Division Report No CE85-2696-K). Hydrogen content of the diets averaged 6.13 $\pm$ 0.17% dry wt (mean $\pm$ SE, n = 9). The hydrogen content of the mice was similar among animals sampled at the beginning and end of the experiment and averaged 6.93 $\pm$ 0.28% dry wt (n = 7). The specific activity of organically bound tritium in diets and mice was calculated by dividing the observed activity by the above average values.

The protein-nitrogen content of diets and mice was determined by the micro-Kjeldahl method of Fels and Veatch (1959), with protein estimated by multiplying the measured nitrogen by 6.25. Protein content of the diets was 27.4 $\pm$ 2.9% dry wt (n = 9), while the protein content of mice averaged 62.9 $\pm$ 2.3% dry wt (n = 7).

Changes in tritium specific activity with time were analyzed using an iterative, non-linear least squares procedure (SAS - Proc NLIN). Selection of a one or two compartment model was based on an examination of residual values and plots of the respective models (Draper and Smith 1981). As non-linear regression methods were employed, the confidence intervals estimated for the models are only approximate. The estimated rates and steady state concentrations were compared using Z tests at the 95% confidence level ($p < 0.05$ in a two-tailed test).

RESULTS

For all treatments, the specific activity of tissue water tritium increased quickly following exposure to tritiated water, diet or both, then decreased rapidly with the termination of tritium exposure (Figure 1). During tritium exposure (Day 0 to 56), changes in activity of both tissue water tritium were described by a single compartment model of the form:

$$Q_{(t)} = Q_{(eq)} (1 - e^{-k_1(t)}) \qquad (1)$$

where $Q_{(t)}$ is the activity (kBq/g H) of the sample at time, t, $Q_{(eq)}$ is the steady state (asymptotic) activity attained by the sample, t is time (in days), and k_1 is the first order rate constant during tritium exposure. (The half-life of tritium in either tissue water or organically bound compartments was determined by dividing 0.693 by the estimated rate constant.) For the period following the termination of tritium exposure, from days 56 to 70 ($56 < t \geq 70$), changes in activity of tissue water tritium were best described by a single compartment clearance model of the form:

$$Q_{(t)} = Q_{(56)} e^{-k_2(t2)} \qquad (2)$$

where $Q_{(56)}$ is the activity of the sample at termination of tritium exposure (day 56) as predicted by equation (1) above, k_2 is the first order rate constant during depuration, t2 is time in days following termination of tritium exposure ($t2 = t - 56$) and other terms as given in equation (1).

The estimated steady state specific activities of tissue water tritium and the rate constants describing tritium accumulation (Table 1) differed significantly among treatments. During tritium exposure, the steady state specific activity of tissue water tritium of mice exposed only to tritiated drinking water was estimated as 15.9 $\pm$ 0.1 kBq/g H, or approximately 70% of the specific activity of the drinking water (22.9 $\pm$ 0.1 kBq/g H). For mice exposed to tritiated drinking water and diet, the steady state specific activity of tissue water tritium (22.8 $\pm$ 0.1 kBq/g H) was not significantly different from the drinking water. The estimated steady state specific activity of tissue water tritium of mice exposed only to tritiated diet (7.04 $\pm$ 0.1 kBq/g H) was approximately 20% of the specific activity of the diet but was orders of magnitude greater than the specific activity of their unlabeled drinking water. Interestingly, the steady state tissue water specific activity of mice exposed to tritiated water and diet was essentially equivalent to the sum of the steady state tissue water specific activity of mice exposed to tritiated water only and that of mice exposed to tritiated diet only.

The rapid exchange of tritium with tissue water was evident in the relatively large values of the estimated rate constants (Table 1), which correspond to tissue water tritium half-lives ranging from 1 to 2.6 d. Both during and after tritium exposure, the rate constants estimated for tissue water tritium in mice exposed to tritiated water only, were significantly greater than the corresponding rate constants estimated for mice exposed to both tritiated water and diet. In addition, the rate constants for both the above treatments were significantly greater than the rate constants of mice exposed to tritiated diet only. For all treatments, rate constants estimated during exposure were slightly but significantly greater than the corresponding rate constant estimated following tritium exposure.

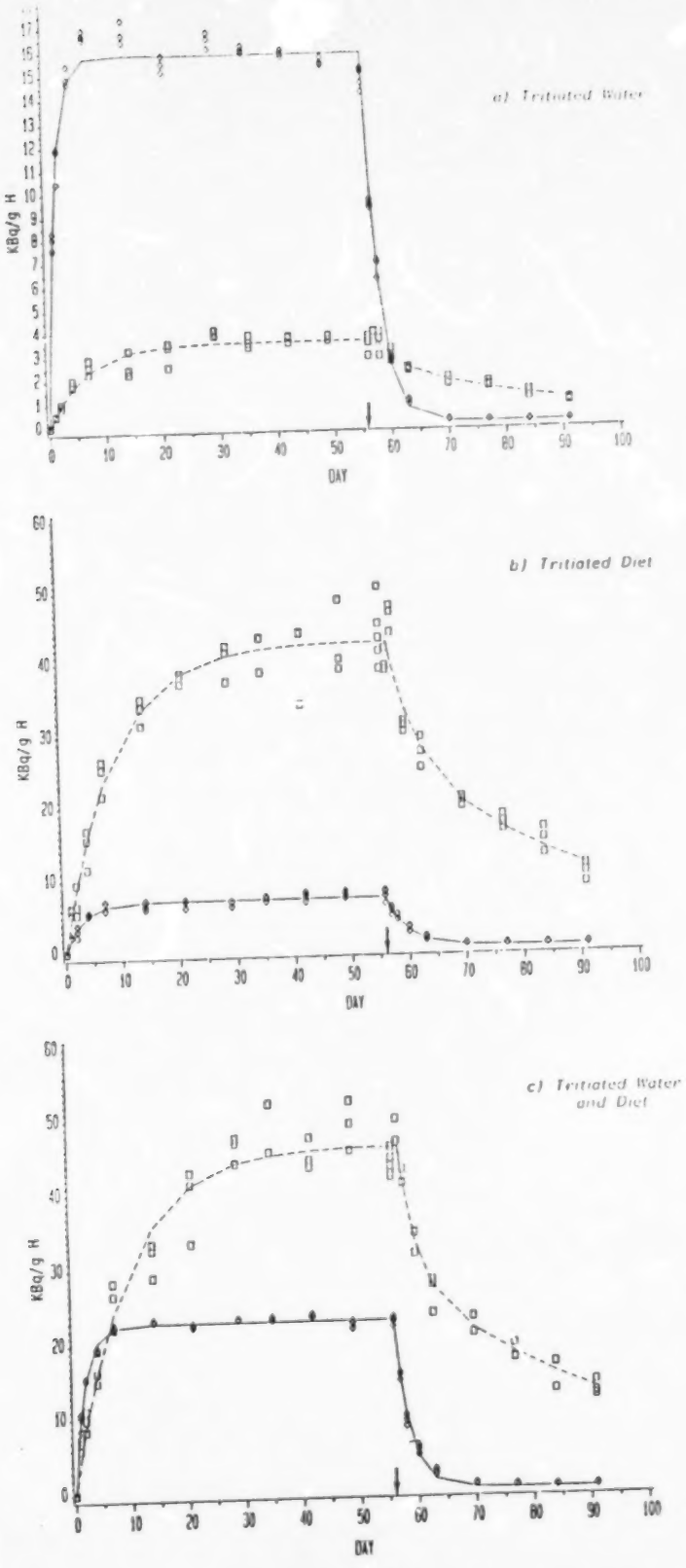

FIGURE 1
SPECIFIC ACTIVITY OF TISSUE WATER TRITIUM (○;——) AND ORGANICALLY BOUND TRITIUM (□;----) OF MICE DURING AND FOLLOWING EXPOSURE TO: a) TRITIATED WATER, b) TRITIATED DIET OR c) TRITIATED WATER AND DIET. LINES WERE PLOTTED BY COMPUTER TO EQUATIONS 1, 2 AND 3 USING VALUES IN TABLES 1 AND 2. TRITIUM EXPOSURE WAS TERMINATED AT DAY 56 (↓).

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Table 1

Estimated Steady State Activity and Rate Constants of Tissue Water Tritium of Mice Exposed to Tritiated Water, Diet or Both (Estimate ± SE)

Tritium Source	Steady State Activity Q(eq) (kBq/g H)	Rate Constant During Exposure k_1 (/d)	Following Exposure k_2 (/d)
Water (22.9 kBq/g H)	5.9 ± 0.1	0.68 ± 0.03	0.46 ± 0.02
Diet (34.4 kkBq/g H)	7.0 ± 0.1	0.34 ± 0.03	0.27 ± 0.02
Both	22.8 ± 0.1	0.55 ± 0.02	0.41 ± 0.01

Table 2

Estimated Steady State Activity and Rate Constants of Organically Bound Tritium of Mice Exposed to Tritiated Water, Diet or Both (Estimate ± SE)

Tritium Source	Compartment Activity			Rate Constant		
	During Exposure	Following Exposure		During Exposure	Following Exposure	
	Q(eq)	$Q_{(2)}$	$Q_{(3)}$	k_1	k_2	k_3
	(kBq/g H)			(/d)		
+Water (22.9 kBq/g H)	3.5 ±0.1	1.6 ±0.5	2.7 ±0.6	0.16 ±0.01	0.31 ±0.23	0.029 ±0.001
Diet (34.4 kBq/g H)	42.6 ±0.8	20.3 ±8.5	27.8 ±9.6	0.11 ±0.01	0.21 ±0.15	0.026 ±0.013
Both	46.1 ±0.8	26.1 ±4.0	29.7 ±4.2	0.11 ±0.01	0.36 ±0.14	0.023 ±0.006

The activity of organically bound tritium increased much less rapidly during tritium exposure

than tissue water tritium but then decreased more slowly on the termination of tritium exposure (Figure 1). During tritium exposure, accumulation of organically bound tritium was best described by a single compartment model similar to that used to describe accumulation of tissue water tritium (Equation 1 above). Following termination of tritium exposure, however, two components were required to best describe the clearance of organically bound tritium. The two components of clearance of organically bound tritium were superimposed in a two compartment model of the form:

$$Q_{(t)} = Q_{(2)}e^{-k_2(t2)} + Q_{(3)}e^{-k_3(t2)} \qquad (3)$$

where $Q_{(2)}$ and $Q_{(3)}$ are the specific activities of the two compartments which are cleared at rates given by the first-order rate constants, k_2 and k_3, respectively.

In contrast to tissue water tritium, diet was the major factor in the accumulation of organically bound tritium, with water providing an additional influence. As observed with tissue water tritium, the steady state activities of organically bound tritium estimated during tritium exposure (Table 2) differed significantly among treatments. During tritium exposure, the steady state specific activity of organically bound tritium of mice exposed to tritiated water alone (3.5 $\pm$ 0.1 kBq/g H) was approximately 22% of the steady state specific activity of tissue water tritium of these mice or 15% of the activity of their drinking water. The steady state specific activities of organically bound tritium attained by mice fed tritiated diet, either alone (42.6 $\pm$ 0.8 kBq/g H) or with tritiated water (46.1 $\pm$ 0.8 kBq/g H) were significantly greater than the specific activity of the diet (34.4 $\pm$ 0.4 kBq/g H). However, when activities were expressed per unit protein, the steady state activities of mice exposed to tritiated diet only (4.69 kBq/ g protein) or tritiated diet and drinking water (5.08 kBq/g protein) were only 61% and 66%, respectively, of the activity of the diet (7.69 kBq/g protein). As was observed with tissue water tritium, the steady state activity of organically bound tritium of mice exposed to tritiated water and diet was approximately equal to the sum of the steady state activities of organically bound tritium of mice exposed to tritiated water only and that of mice exposed to tritiated diet only.

The contrast between the dynamics of tissue water tritium and organically bound tritium was also apparent in the estimated rate constants. During tritium exposure, when the kinetics of organically bound tritium were adequately described by a single compartment model, the rate constants for each treatment were significantly smaller than the corresponding tissue water tritium rate constant. The rate constant for accumulation of organically bound tritium of mice during exposure to tritiated water only (0.16 $\pm$ 0.01, half-life - 4.3 d) was significantly greater than the rate constants of mice during exposure to tritiated diet alone (0.11 $\pm$ 0.01, half-life - 6.3 d) or tritiated diet and water (0.11 $\pm$ 0.01, half-life - 6.3 d). The latter rate constants did not differ significantly.

The loss of organically bound tritium from the mice following tritium exposure was best described by a two compartment model, in which approximately 40% of the organically bound tritium was cleared rapidly, with half-lives of 2.0 to 3.2 d, while the remaining organically bound tritium was cleared much more slowly, with half-lives 24 to 30 days. For all treatments, the combined totals of the coefficients of the fast and slow clearing pools of organically bound tritium ($Q_{(2)}$+$Q_{(3)}$) were consistently greater than the coefficient of organically bound tritium of the single compartment model during tritium exposure (Q(eq)). However, the differences between

the coefficients of the two periods were within the range of errors associated with these estimates and were not statistically significant. The rate constants estimated for the faster clearing compartment of organically bound tritium did not differ significantly among treatments. In addition, these rate constants were not significantly different from the rate constants estimated for tissue water tritium for the corresponding treatment. Rate constants of the slower clearing compartment of organically bound tritium were significantly different from rate constants for the faster clearing compartment but did not differ significantly among treatments.

DISCUSSION

The changes observed in the tritium specific activity of the mice reflected the dynamic equilibrium of three principal compartments - tissue water tritium, loosely bound or exchangeable organically bound tritium, and tightly bound, non-exchangeable organically bound tritium. During tritium exposure, the effects of water and diet on the steady state activities of tissue water tritium and organically bound tritium were largely independent and additive. The steady state activities of tissue water tritium and organically bound tritium in mice exposed to both tritiated water and diet were essentially equivalent to the sum of the steady state specific activities of mice exposed only to tritiated water and that of mice exposed only to tritiated diet. Tissue water tritium was accumulated fairly rapidly, with half-lives of 1 to 2.6 d. In contrast, organically bound tritium was accumulated more slowly. Following tritium exposure, the loss of organically bound tritium was resolved into fast and slow clearance compartments. The rate constants for the fast clearing organically bound tritium compartment were not significantly different from the corresponding rate constant for tissue water tritium, and thus likely represented the clearance of loosely bound or exchangeable organically bound tritium. The second organically bound tritium compartment was cleared much more slowly, with half-lives of 20 to 30 d, and likely represented non-exchangeable tritium.

For all treatments, the specific activity of tissue water tritium of the mice increased rapidly after initiation of tritium exposure. Drinking water and food were essentially independent and additive in their contribution to the specific activity of tissue water tritium of the mice as the steady state tissue water specific activity of mice exposed to tritiated water and diet (22.8 kBq/g H) was essentially equivalent to the sum of the steady state tissue water specific activity of mice exposed to tritiated water only (15.9 kBq g H) and that of mice exposed to tritiated diet only (7.04 kBq/g H). In mice exposed to tritiated water only, tissue water tritium equilibrated at approximately 70% the specific activity of the drinking water. This value was similar to that observed in earlier studies (Thompson and Ballou 1956; Hatch and Mazrimas 1972), where the difference between the steady state activity of tissue water tritium and drinking water in these earlier studies was attributed to a dilution of the tissue water pool within the organism by metabolic and food water (Hatch and Mazrimas 1972). As the mice were fed a dried diet, food water should have contributed relatively little to tissue water tritium in this study. Conversely metabolic and exchange water would be the sources of the activity of tissue water tritium in mice fed tritiated diet only.

The half-lives of tissue water tritium of mice exposed only to tritiated water (1 d during exposure, 1.5 d after exposure) were in agreement with the value of 1.13 ± 0.14 d previously reported for mice after a single injection of tritiated water (Richmond et al. 1962). As the rate of turnover of body water in mammals is inversely related to body size (Richmond et al. 1962), the growth of the mice (from approximately 24 g at the beginning of tritium exposure to approximately 31 g at the end of exposure) may account for the slight but significant decrease in

rate constants for tissue water tritium following tritium exposure.

The differences among treatments of rate constants for tissue water tritium likely resulted from differences in the rates of adsorption and distribution of the sources of tritium exposure. The largest rate constant and shortest half-life of tissue water tritium was observed with mice exposed only to tritiated water, while the smallest rate constant and longest half-life were obtained with mice exposed only to tritiated diet. These results are consistent with the more rapid adsorption of ingested water relative to dietary amino acids (Prosser 1973). As both water and diet contributed to the tissue water tritium of mice exposed to tritiated water and diet, rate constants for these animals were intermediate relative to mice exposed to either tritium source alone.

As observed for tissue water tritium, diet and drinking water appeared to be largely independent and additive in their contribution to the specific activity of organically bound tritium in mice. During tritium exposure, the steady state activity of organically bound tritium of mice exposed to tritiated water and diet (46.1 $\pm$ 0.75 kBq/g H) was approximately equal to the sum of the steady state activities of organically bound tritium of mice exposed to tritiated water only (3.52 $\pm$ 0.07 kBq/ g H) and that of mice exposed to tritiated diet only (42.6 $\pm$ 0.83 kBq/ g H). The lower steady state specific activity of organically bound tritium of mice fed tritiated diet alone, relative to mice exposed to tritiated diet and water likely resulted from continuous metabolic conversion of tritium between exchangeable and non-exchangeable pools and wash-out of exchangeable tritium.

During tritium exposure, the organically bound tritium fraction of mice exposed only to tritiated water reached a steady state activity which was approximately 22% of the activity of their tissue water tritium. This value corresponds well with observations that from 20 to 30% of the hydrogens of most tissue compounds was derived from tissue water in rats and mice exposed to tritiated water and fed non-tritiated diets (Thompson and Ballou 1956; Hatch and Mazrimas 1972) and similar values obtained for trout and other aquatic organisms reared in tritiated water but fed non-tritiated diets (Bruner 1973; Komatsu et al. 1981; Ontario Hydro Research Division Report No 84-217-K). Similarly, Siri and Evers (1962) estimated that approximately 30% of the hydrogen in lean tissue solids is exchangeable with water, although the exact quantity of hydrogen available for exchange will be contingent on the specific composition of the organic material. Metabolic incorporation of tritium in addition to exchange may thus be required to achieve specific activities greatly in excess of 30% of the ambient water.

The pronounced influence of diet on the accumulation of organically bound tritium was evident in the elevated steady state activity of organically bound tritium attained by mice fed tritiated diet, either alone or in combination with tritiated water. These results were in agreement with earlier studies where tritium activities in the organically bound tritium fraction of rats or rabbits exposed to organically bound tritium in the diet were significantly greater than the activities of tissues of animals exposed for the same duration to tritiated water only (Rochalska and Szot 1977; Pietrzak-Flis et al. 1978). The high retention of tritiated amino acids in the diet is consistent with conclusions of Commerford et al (1983) and Commerford (1984) that nearly all of the non-exchangeable hydrogen in essential amino acids is transferred intact from the diet. Amino acids which are essential to mice (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine (Maddy and Elvehjem 1949)) comprised approximately half of the activity of the tritiated amino acid mixture used in this experiment.

The present results demonstrate that mice consuming food with an organically bound tritium

specific activity significantly higher than the ambient water, will maintain tissue specific activities of organically bound tritium which are significantly greater than the ambient water. Similarly, in earlier studies, rainbow trout fed tritiated diets maintained specific activities of organically bound tritium which were significantly greater than the ambient water (Ontario Hydro Research Division Report No 84-217-K). These results are in agreement with studies of Estep and Dabrowski (1980), in which the ratio of deuterium to hydrogen in organically bound hydrogen of both mice and marine snails, Littorina obtusata, was principally determined by diet rather than ambient water. As the steady state activity attained by the mice after chronic exposure to tritiated water or diet was less than or equal to the the activity of the source (water (Bq/g H) or diet (Bq/g protein)), these results were also consistent with the use of a concentration coefficient of unity as a conservative value for models of the environmental tritium (Vanderplong et al 1975). Published reports of organically bound tritium specific activities significantly greater than the tissue water in animals in contaminated natural environments (Martin and Koranda 1972; Kirchmann et al 1979) may indicate that these animals consumed a diet with a high specific activity of organically bound tritium relative to the ambient water.

For all treatments, the accumulation of organically bound tritium was best described by a single compartment model during tritium exposure and a two compartment model following tritium exposure. The rate constants for the single compartment model were intermediate relative to the fast and slow rate constants of the two compartment model and the continuing input of tritium during exposure may have obscured the two components of tritium elimination.

Following tritium exposure, the rate constants for the fast clearing compartment (half-lives of 2 to 3 d) did not differ significantly among treatments, nor were these rate constants significantly different from the rate constant for simultaneous loss of tissue water tritium from the mice. As the rate constants of this fast clearing compartment were not significantly different from the rate constants for tissue water tritium, the fast clearing compartment likely represented clearance of loosely bound or exchangeable organically bound tritium from the mice. The remaining organically bound tritium was cleared more slowly, with half-lives of 24 to 30 d. These values are in general agreement with a half-life of approximately 22 d estimated for the shorter lived components of the carcass of the rat (Thompson and Ballou 1956). Additional slower clearing components may have been resolved if the experiment had been extended. Thompson and Ballou (1956) reported a half-life of 130 d for the longer lived component of rat carcass, while some of the organically bound tritium in collagen was essentially inert, with an estimated half-life of 1000 d. As cumulative dose calculations are integrated over time, these slowly cleared components of the organically bound tritium pool may be particularly critical in determining the total dose commitment from chronic exposure to environmental tritium.

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APPENDIX 1

Composition of Tritiated Amino Acid Mixture (Amersham Trk. 440-Batch 43; Batch Analysis Sheet H/8343

Amino Acids	Composition by Activity (1 MBq of Mixture Content)	Specific Activity of Component Amino Acids
L-[2,3-^{3}H] Alanine	95 KBq	2.22 TBq/mmoL
L-[5-^{3}H] Arginine (E)	70 KBq	703 GBq/mmoL
L-[2,3-^{3}H] Aspartic Acid	100 KBq	518 GBq/mmoL
L-[G-^{3}H] Glutamic Acid	90 KBq	814 GBq/mmoL
[2-^{3}H] Glycine	60 KBq	370 GBq/mmoL
L-[2,5-^{3}H] Histidine (E)	15 KBq	1.89 TBq/mmoL
L-[4,5-^{3}H] Isoleucine (E)	60 KBq	4.03 TBq/mmoL
L-[4,5-^{3}] Leucine (E)	125 KBq	1.67 TBq/mmoL
L-[4,5-H] Lysine (E)	50 KBq	1.48 TBq/mmoL
L-[4-^{3}H] Phenylalanine (E)	75 KBq	9.25 GBq/mmoL
L-[3,,4(n)-^{3}H] Proline	55 KBq	1.92 TBq/mmoL
L-[3-^{3}H] Serine	30 KBq	481 GBq/mmoL
L-[G-^{3}H] Theronine (E)	55 KBq	555 GBq/mmoL
L-[G-^{3}H] Tyrosine (E)	60 KBq	1.59 TBq/mmoL
L-[33,4(n)-^{3}H] Valine (E)	60 KBq	1.11 TBq/mmoL

E - Essential Amino Acid for Mice (Maddy and Eluehjen 1949)